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Structure of the BH Domain from Graf and its Implications for Rho GTPase Recognition

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ABSTRACT: Cellular signaling by small G-proteins is down-regulated by GTPase-activating proteins (GAPs), which increase the rate of GTP hydrolysis. The GTPase regulator associated with focal adhesion kinase (Graf) exhibits GAP activity toward the RhoA and Cdc42 GTPases, but is only weakly active toward the closely related Rac1. We determined the crystal structure of a 231-residue fragment of Graf (GrafGAP), a domain containing the GAP activity, at 2.4-Å resolution. The structure clarifies the boundaries of the functional domain and yields insight to the mechanism of substrate recognition. Modeling its interaction with substrate suggested that a favorable interaction with Glu-95 of Cdc42 (Glu-97 of RhoA) would be absent with the corresponding Ala-95 of Rac1. Indeed, GrafGAP activity is diminished approximately 40-fold toward a Cdc42 E95A mutant, whereas a approximately 10-fold increase is observed for a Rac1 A95E mutant. The GrafGAP epitope that apparently interacts with Glu-95 (Glu-97) contains Asn-225, which was recently found mutated in some myeloid leukemia patients. We conclude that position 95 of the GTPase is an important determinant for GrafGAP specificity in cellular function and tumor suppression.